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Supplemental Information

The c-di-GMP Binding Protein YcgR Controls Flagellar Motor Direction and Speed to Affect Chemotaxis by a "Backstop Brake" Mechanism

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Media, Growth conditions, Cloning and Mutagenesis. Procedures for transformation and plasmid isolation were described previously (Tang and Blair, 1995). Bacteria were grown in L-broth (LB) containing 10 g tryptone, 5 g Yeast extract and 5 g NaCl per liter. Antibiotics used in this study are ampicillin (100μg/ml), chloramphenicol (20μg/ml), and kanamycin (50μg/ml). P22 transduction was utilized to construct mutant combinations in *S. enterica*. Excision of KAN cassettes was achieved by expression of the FLP recombinase encoded on pCP20 (Datsenko and Wanner 2000).

For basic cloning and gene expression from a plasmid, gene sequences were amplified using PCR from the genomic DNA of wild-type *S. enterica* and cloned into pBAD24 (*ycgR*) or pBAD33 (*fliM*, *fliG*). Both cloning vectors are inducible with L-arabinose and have been previously described (Guzman et al., 1995). Specific mutations in *ycgR*, *fliM*, and *fliG*, and *gfp* fusion to the C-terminus of *ycgR* were constructed using over-lap extension PCR as previously described (Saiki et al., 1988), or using the Quick Change protocol (Stratagene, La Jolla, CA). Mutations were confirmed by DNA sequencing.

Motility assays. LB swarm plates were made using 0.6% Eiken agar (Eiken Chemical, Tokyo, Japan) and also were supplemented with 0.5% glucose (Wang et al., 2004). In the case of *ycgR* over-expression in *Salmonella*, 0.5% L-arabinose was substituted for glucose; this sugar supports swarming as efficiently as glucose (Harshey and Matsuyama, 1994). Swim plates were made using 0.3% bacto agar. *E.coli ycgR* were over-expressed using either IPTG (isopropyl-b -D-thiogalactopyranoside) or sodium salicylate as noted in the corresponding figure legends. *Salmonella ycgR* were over-expressed using 0.2% L-arabinose. Antibiotics used in the motility plates are either ampicillin (50μg/ml) or chloramphenicol (50μg/ml) or both.

To measure the effect of over-expressed MotA, MotB, MotAB, or CheY on the motility of the Δ*yhjH* or other motility-impaired strains, cells were transformed with the corresponding plasmids (listed in Supplementary Table 1), and induced with indole acrylic acid or arabinose. Controls were transformed with the parent vectors (pKG116, pTM30 or pRR48). Swimming was measured in plates containing appropriate antibiotics, and inducer(s) at the concentrations indicated in the corresponding figures.

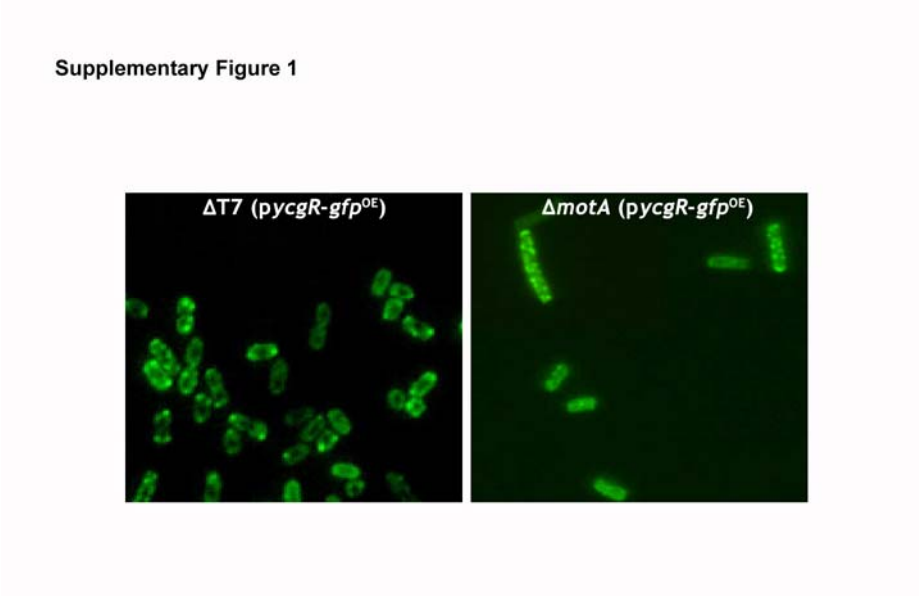

Figure S1. YcgR localization in a *Salmonella* strain deleted for all chemoreceptors, and an *E. coli* strain deleted for MotA. YcgR-GFP expression was induced with arabinose in *Salmonella* strain SM162 (Δ7T) and *E. coli* strain RP6666 (Δ*motA*), and examined by fluorescence microscopy.

Supplementary Figure 2

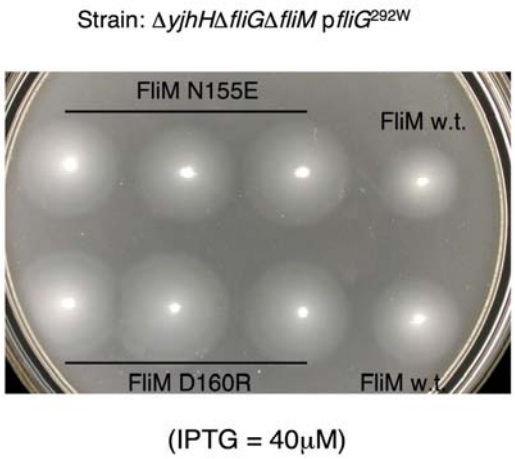

Figure S2. Swimming phenotype of *E. coli* FliM mutants. Plasmids expressing wild-type FliM (pDB94.A) or its mutant variants (N155E and D160R) were transformed into the Δ*yhjH*Δ*fliG*Δ*fliM* strain along with a second plasmid (pHT53.N292W) expressing FliG with the N292W mutation. Fresh transformants were picked onto swim plates containing tryptone, 40μM IPTG, and 0.27% agar, and plates were incubated at 32˚C for 9-10 h.

Supplementary Figure 3

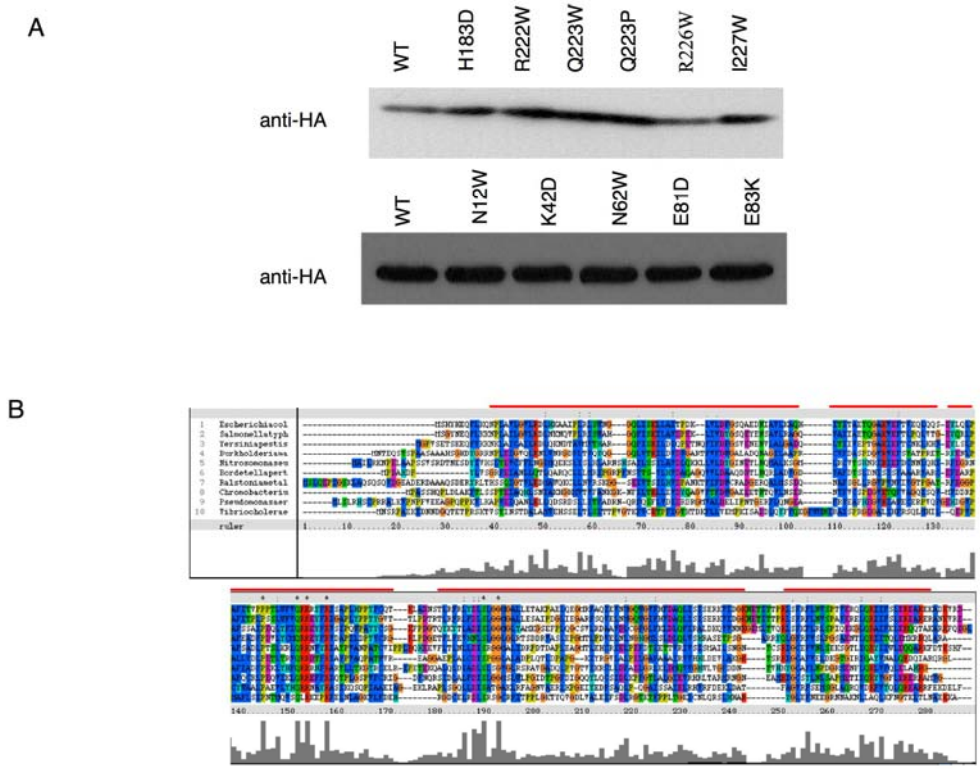

Figure S3. Expression profile of *E. coli* YcgR mutants and multiple alignment of YcgR protein. (A) Anti-HA immunoblots demonstrating the stability of HA-tagged YcgR proteins with the mutations indicated. (B) Alignment of YcgR sequences from several species. Segments included in the homology model of the *E. coli* protein are indicated by the red lines above the alignment.

Supplementary Figure 4

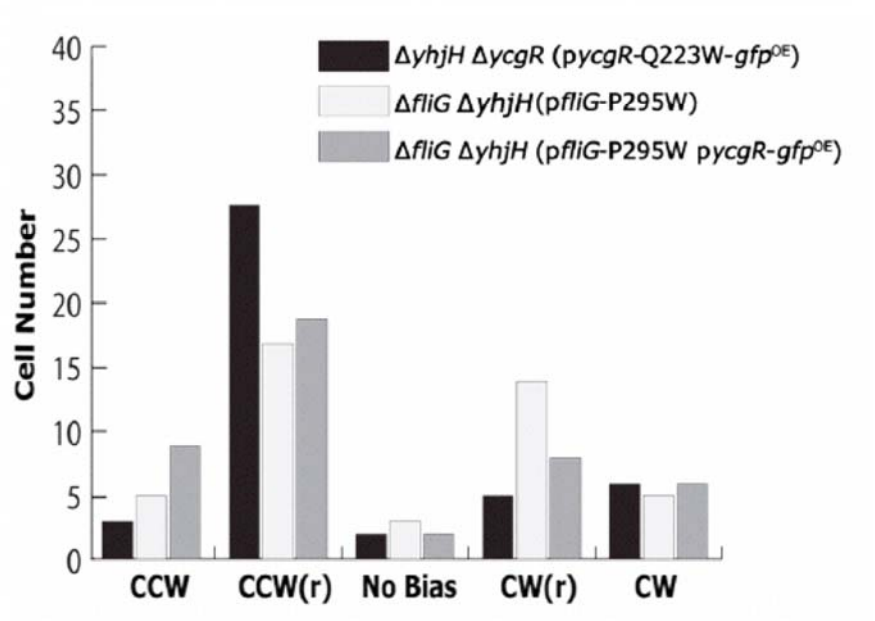

Figure S4. Flagellar rotational bias and effects of the YcgR mutation Q223W and the FliG mutation P295W in *Salmonella*.

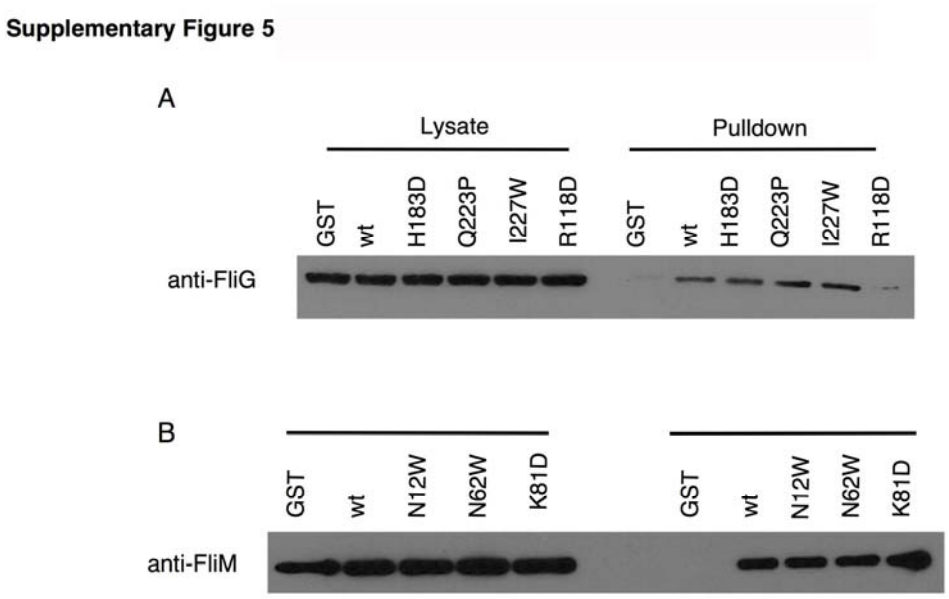

Figure S5. Interaction of *E. coli* YcgR mutants with FliG (panel A**) and FliM (**panel B**).** Pulldown experiments were carried out as described in Experimental Procedures, using a GST-YcgR fusion, and co-isolated FliG and FliM were detected on immunoblots. Samples inclucded c-di-GMP at a concentration of 5 μM. Lysate controls show the levels of protein in samples prior to treatment with the beads.

Table S1. Strains and Plasmids

Strain or Plasmid	Relevant genotype or properties	Source
E. coli		
RP3098	Δ*flhDC*; flagellar genes not expressed	J.S. Parkinson
RP6666	Δ*motA1214*	J.S. Parkinson
DB228	Δ*fliM* null strain	Tang *et al.* (1995)
MS296	wild-type *E.coli* strain	Ko *et al.* (2000)
DB247	Δ*fliG*Δ*fliM*	This study
MS1279	Δ*ycgR* (*ycgR*:: FRT-FRT) in MS296	This study
MS1280	Δ*yhjH* (*yhjH*:: FRT-FRT) in MS296	This study
MS1467	Δ*ycgR*Δ*yhjH* (*ycgR*:: TetRA) in MS1280	This study
MS2234	Δ*fliM*Δ*yhjH* (*yhjH*:: TetRA) in DB228	This study
MS2303	Δ*fliG*Δ*fliM*Δ*yhjH* (*yhjH*:: TetRA) in DB247	This study
Salmonella enterica		
14028	Wild-type ATCC Strain	Mariconda *et al.* (2006)
QW108	*yhjH*:: KAN	Q. Wang
QW140	Δ*fliG*	Q. Wang
QW165	*ycgR*:: KAN	Q. Wang
QW182	Δ*fliM*	Q. Wang
QW262	Δ*yhjH*	Wang *et al*. (2006)
QW278	Δ*ycgR*	Wang *et al*. (2006)
SM162	deleted of all 7 chemotaxis receptors (Δ7T)	S. Mariconda
SM380	Δ*cheZ*	S. Mariconda
SM387	Δ*cheB*	S. Mariconda
VN10	14028 (p*ycgR*)	This Study
VN11	Δ*yhjH* (p*ycgR*)	This Study
VN29	Δ*cheB yhjH*:: KAN (p*ycgR*)	This Study
VN33	Δ*cheZ* Δ*yhjH* (p*ycgR*)	This Study
VN45	14028 (p*ycgR-gfp*)	This Study
VN46	Δ*yhjH* (p*ycgR-gfp*)	This Study
VN47	Δ7T (p*ycgR-gfp*)	This Study
VN50	Δ*fliM yhjH*:: KAN (p*ycgR-gfp*)	This Study
VN55	Δ*yhjH* Δ*ycgR*	This Study
VN67	Δ*yhjH* Δ*ycgR* (p*ycgR-gfp*)	This Study
VN70	Δ*yhjH* ΔycgR (p*ycgR*-R118D-*gfp*)	This Study
VN71	Δ*fliM* (p*fliM*)	This Study
VN73	Δ*fliM yhjH*:: KAN (p*fliM*)	This Study
VN78	Δ*fliM yhjH*:: KAN (p*fliM*-N155E)	This Study
VN79	Δ*fliM yhjH*:: KAN (p*fliM*-L160E)	This Study
VN80	Δ*fliM yhjH*:: KAN (p*fliM*-N155E; p*ycgR-gfp*)	This Study
VN81	Δ*fliM yhjH*:: KAN (p*fliM*-L160E; p*ycgR-gfp*)	This Study

VN82	Δ*yhjH* Δ*ycgR* (p*ycgR*-Q223W)	This Study
VN83	Δ*fliG* (p*fliG*)	This Study
VN84	Δ*fliG* (p*fliG*-P295W)	This Study
VN85	Δ*fliG yhjH*:: KAN (p*fliG*-P295W)	This Study
VN86	Δ*fliG yhjH*:: KAN (p*fliG*-P295W; p*ycgR-gfp*)	This Study
VN88	*fliN*:: KAN (p*ycgR-gfp*)	This Study
VN89	Δ*yhjH fliN*:: KAN (p*ycgR-gfp*)	This Study
VN90	Δ*motA yhjH*:: KAN (p*ycgR-gfp*)	This Study
Plasmid or Phage		
pCP20	FLP Recombinase	Datsenko & Wanner (2006)
pKD4	Kanamycin Template	Datsenko & Wanner (2006)
pKD46	λ Red Recombinase	Datsenko & Wanner (2006)
pBAD24	Cloning Vector; Ap^R	Guzman *et al.* (1995)
pBAD33	Cloning Vector; Cm^R	Guzman *et al.* (1995)
pRR48	P*tac* expression vector, Ap^R	J.S. Parkinson
pKG116	salicylate-inducible expression vector, Cm^R	J.S. Parkinson
pTM30	Ptac expression vector, Ap^R	Paul *et al.* (2006)
pHT39	FliN expression vector	Lloyd *et al.* (1996)
pHT100	GST-only (negative control) vector	Tang *et al.* (1996)
pHT53	FliG expression vector	Tang *et al.* (1996)
pLW3.33	P*trp-motB*, Ap^R	Zhou *et al.* (1998)
pDB45	P*trp-motAmotB*, Ap^R,	Zhou *et al.* (1998)
pDB66	P*ara-cheY*, Cm^R	Paul *et al.* (2006)
pDB94.A	P*tac-fliM*, Cm^R	This study
pGM1	P*tac-motB*, Ap^R	Zhou *et al.* (1998)
pSB1	P*T7-motA*$_{70\text{-}170}$, Ap^R	This study
pKP41	*fliN* in pKG116	This study
pKP99	*ycgR* in pKG116	This study
pKP198	*fliL* in pKG116	This study
pKP203	*ycgR* in pRR48	This study
pKP232	*ycgR*^{R118D} in pKG116	This study
pKP233	*ycgR*-HA tag in pRR48	This study
pKP234	*ycgR*^{R118D}-HA tag in pRR48	This study
pKP266	*ycgR*-HA tag in pHT100	This study
pKP283	*ycgR*^{R118D}-HA tag in pHT100	This study
pKP298	*fliG*^{297C}-HA tag in pKG116	This study
pKP299	*fliG*$^{117+166C}$- HA tag in pKG116	This study
pKP347	*ycgR*-HA tag in pKG116	This study
VNP5	p*ycgR* cloned in pBAD24	This study
VNP8	p*ycgR-gfp* in pBAD24	This Study
VNP11	p*ycgR*-R118D-*gfp* in pBAD24	This Study
VNP14	p*fliM* in pBAD33	This Study
VNP18	p*fliM*-N155E in pBAD33	This Study

VNP19	p*fliM*-L160E in pBAD33	This Study
VNP20	p*ycgR-gfp* in pBAD33	This Study
VNP22	p*ycgR*-Q223W in pBAD24	This Study
VNP24	p*fliG* in pBAD33	This Study
VNP27	p*fliG*-P295W in pBAD33	This Study
Phage P22	HT12/4int103	Mariconda *et al.* (2006)

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